

SDS-PAGE

Preparation of Bacterial Cells

1. Inoculate the overnight culture into sterilized tubes or conical flasks with LB medium (with corresponding antibiotics)

Incubate with shaking(200rpm) at 37°C until the cell cultures have a O.D.600 of 0.5~0.6.

2. Divide the cultures into two sterilized tubes or conical flasks. One add inducer (IPTG) to a final concentration of 0.8mM. The other serves as control. Continue incubating in shaker overnight, and adjust the temperature and motion to 20°C, 80rpm.

3. Harvest the bacteria by centrifugation at 4°C, 4000rpm for 5 min, discard the supernatants and use PBS(pH 7.2) to wash and resuspend the cells for several times.

Bacterial Cell Lysis

1. Resuspended cells undergo 2-3 rounds of freezing in liquid nitrogen followed by thawing in a 2°C waterbath. Transfer the cells into a small conical flask.

2. Set the flask in ice, make sure the liquid was completely surrounded by ice. Then disrupt the cells by sonification for 45 cycles involving 15 sec of sonification and interval 5 sec cooling in ice.

3. Centrifuge the cell lysates at 4 °C , 11000rpm for 5 min, discard the pellets, the supernatants as the protein samples for sample loading.

Electrophoresis

1. Preparing acrylamide gels

For 1 piece of gel (6×8×0.1cm ³)	Separating gel solution	Stacking gel solution
A	3.3ml	0.67ml
B	2.5ml	0
C	0	1ml
dH ₂ O	4.2ml	2.3ml
10% APS	50ul	30ul
TEMED	5ul	5ul

A (acrylamide stock solution):
30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide
B (4×separating gel buffer) (100ml):
75ml 2M Tris-HCl (pH 8.8)
4ml 10% (w/v) SDS
21ml dH₂O
C(4×stacking gel buffer) (100ml):
50ml 1M Tris-HCl (pH 6.8)
4ml 10% (w/v) SDS
46ml dH₂O

1).Inject the separating gel solution between two settled glass plate first, and add dH₂O about the height of 1 cm.

2).After polymerization reaction is done, suck up the water upon

the separating gel. Then inject the stocking gel solution upon the separating gel and insert the comb.

3). Remove the comb after polymerization reaction is done.

2. Resemble the gel cassette and the power source, load the $1 \times$ electrophoresis buffer in the tank till the sample wells are submerged.

$1 \times$ electrophoresis buffer(1L):

3g Tris base (25mM)

14.4g glycine (192mM)

1g SDS (w/v=0.1%)

Add dH₂O to a final volume of 1L

Adjust pH to 8.3

3. Samples loading:

$5 \times$ protein loading buffer(10ml):

0.6ml 1M Tris-HCl (pH 6.8)

5ml 50% (v/v) glycerol

2ml 10% (w/v) SDS

0.5ml 2-mercaptoethanol (14.4mM)

1ml 1% (w/v) bromophenol blue

0.9ml dH₂O

1). Mix the protein samples with $5 \times$ protein loading buffer.

2). Denature the mixture for 5 min at 100°C followed by ice-bath.

3). Load it in to the sample wells (15ul per well, 5ul for marker).

4. Turn on the power, undergo the electrophoresis at 80V, and adjust the voltage to 120V when the bromophenol blue indicator runs down into the separating gel.

5. Stain the protein gel with coomassie blue solution for 15 min.

Coomassie blue solution(1L):

1.0g brilliant blue R-250

450ml methanol

450ml dH₂O

100ml Glacial Acetic Acid

6. Destain the protein gel with destaining solution until the background coloration disappears.

Destaining solution(1L):

100ml methanol

800ml dH₂O

100ml Glacial Acetic Acid

7. Photograph on the scanner.